Developing ChemFin™, a Miniature Biogeochemical Sensor Payload for Gliders, Profilers, and other AUVs

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LONG-TERM GOALS

The first goal of this project involves the further development and transition of ChemFIN™, a prototype autonomous profiling sensor for chemicals and biomolecules, into a commercial product that can be readily deployed on fixed or mobile ocean observation platforms such as coastal gliders, profiling moorings, and propeller driven unmanned underwater vehicles (UUVs). The second goal of this project is to integrate a flow immunosensor technology (i.e. biosensor), developed by researchers at the Naval Research Laboratory, into ChemFIN for the detection of biomolecules of interest, such as specific biotoxins (i.e saxitoxin) that are released during harmful algal blooms (HABs). ChemFIN is being developed for sustained, autonomous ocean observations of specific chemical and biochemical distributions and spatial and temporal variability. ChemFIN is an evolving compact sensor payload, utilizing microfluidics, and is particularly designed for "low-power" underway measurements on gliders, propeller-driven autonomous underwater vehicles (AUVs) and autonomous profilers.

OBJECTIVES

The first objective is to use recent advances in micro-fluidics and optical detectors to improve the ChemFIN sensor. The technical improvements involve reducing sample flow rates and volumes and thus reagent and power consumption, extending the length of field deployments by developing new technologies to suppress bio-fouling, increasing the ease of use by simplifying operation, pre-packaging reagents and thoroughly documenting the performance by conducting demonstration experiments in coastal waters. The second objective is to adapt and integrate the flow immunosensor analytical technology, developed by NRL researchers, into the MarChem and ultimately the ChemFIN sensor payloads.

APPROACH AND WORKPLAN

Report Documentation Page

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These objectives are being achieved through a partnership between industry (Alfred Hanson, SubChem Systems, Inc.) and government (Anne Kusterbeck, NRL). The two partners have prior experience working together to develop and test new biological/chemical sensing and deployment systems. During this project, the industry partner are taking the lead in developing and testing the commercial versions of the MarChem and ChemFIN sensor payloads while the government partner are taking the lead in the development of alternative analytical technologies for the flow immunosensor application and assistance with the testing and performance evaluation.

WORK COMPLETED

Developmental testing was continued this year by SubChem Systems on their newest new submersible chemical analyzer. ChemFIN^{TM} is a small independent sensor payload, utilizing microfluidics, and is particularly designed for "low-power" underway measurements on gliders, propeller-driven AUVs, autonomous profilers and moorings. During this report period, SubChem engineers further tested and evaluated their new "commercial product" ChemFIN (Figure 1). The ChemFIN was configured for the measurement of nitrate and/or nitrite. Laboratory and field experiments were conducted to evaluate the analytical capability for the ChemFIN to measure and monitor low concentration levels of nitrate and nitrite. The performance of the ChemFIN nitrate analyzer was inter-compared with both the SUNA (Satlantic) and APNA (SubChem) nitrate analyzers (Figure 2).

Collaborative design work and info-exchange discussions between SubChem and NRL also continued on the integration of the NRL Flow Immunosensor technology into the MarChem Analyzer (Figure 3). NRL efforts continued to focus on testing specialized microfluidic coupons for high sample throughput in the immunosensor payload. NRL scientists and SubChem Systems engineers and chemists spent a week working together in RI during September 2011, deploying the MarChem immunosensor payload on the Pier at the URI Graduate School of Oceanography, in Narragansett Bay (Figure 3). Work also continued at NRL to identify and synthesize the appropriate toxin molecules, fluorophore labeled conjugates and antibody combinations needed for the sensor assays. The NRL scientists were able to demonstrate real time biosensor determination of Saxitoxin (Figure 4) using the MarChem Immunisensor.

RESULTS

1. ChemFIN Design, Development and Commercialization Summary: The SubChem Systems ChemFIN design originates from several years of research and development performed under funding through the Office of Naval Research (ONR) and the National Ocean Partnership Program (NOPP). The company has worked to miniaturize components to substantially lessen reagent consumption and power requirements through novel approaches in micro-fluidic components. The ChemFIN features the latest in micro-fluidic advancements from SubChem Systems for the purpose of both long term intermittent sampling and continuous low power profiling on autonomous profilers, unmanned underwater vehicles, and moorings

The ChemFIN (**Figure 1**) is SubChem System's answer to a requirement for a single or dual channel nutrient sensor with the ability to perform intermittent as well as continuous unattended long term sampling. The ChemFIN is essentially a single or dual channel APNA but with additional enhancements for lowering sample flow and power. The design features a novel micro-fluidic manifold decreasing not only sample volume and flow rate, but cost as well. Where many components

were used to construct the basis of the fluidic system in an APNA, the CHEMFIN has one manifold with a series of micro-fluidic paths and ports. Many legacy components from the multi-channel APNA have been eliminated in the streamlined ChemFIN design reducing cost and production time. The overall system flow rate can now be reduced to 0.25~1.0 ml/min depending on application. Likewise reagent consumption is lessened allowing for an increase in sampling endurance. One of the bigger achievements with the ChemFIN design is the use of a novel insulated reaction path and heating element. This enables the ChemFIN to operate at minimum power penalty for heating the sample flow path. While an APNA may draw up to 30W once heating has stabilized, the ChemFIN will draw only 3W average.

The ChemFIN Nitrate Analyzer uses a chemical analytical procedure that measures both dissolved nitrate (NO_3^{-2}) and dissolved nitrite (NO_2^{-2}), so the concentration of both of these chemicals would be reported simultaneously(Grassoff, 1983).

The new ChemFIN product from SubChem Systems is specifically targeting a market for single or dual channel nutrient sensors. The product is designed for easy integration with commercial CTD systems (i.e. SeaBird Electronics) use in fixed moored applications, vertical profiling moorings, and applications for continuous sampling on unmanned underwater vehicles.

2. MarChem NRL biosensor prototype - real time determination of Saxitoxin: Saxitoxin ((3aS-(3a-α,4-α,10aR*))2,6-diamino-4-(((amino-carbonyl)oxy) methyl)-3a,4,8,9-tetrahydro-1H,10H-pyrrolo(1,2-c)purine-10,10-diol) is the cause of paralytic shellfish poisoning (PSP) and derives its name from the source from which it was originally isolated (i.e. *Saxidomus giganteous*). It was later determined that the toxin actually originated from the dinoflagellate responsible for red tides, *Gonyaulax catenella*. Saxitoxin can be considered a chemical marker for the red tide. As part of this project we proposed to produce a new sensor for this toxin that could be integrated into our underwater sensor.
Using mouse monoclonal antibodies and fluorescently labeled saxitoxin we prepared a coupon for use in our flow immunosensor. All assays were performed in PBS with 0.01% Tween-20 at a flow rate of 0.1 ml/min. Saxitoxin and fluorescently labeled saxitoxin were supplied by Dr. S. Hall (U.S. FDA, College Park, MD). Anti-saxitoxin monoclonal antibodies were supplied by Dr. E. Maertlbauer (University of Munich, Munich, Germany).

For the biosensor displacement immunoassay, fluorescence was monitored using an excitation wavelength of 632 nm and an emission wavelength of 665 nm. The coupon was challenged with discrete doses of the toxin and the fluorescent response recorded (Figure 4, left). Integrating the fluorescent response produced the response curve shown in Figure 4 (right). From this data it is clear that a linear relationship exists between saxitoxin concentration and sensor response. It should be noted that the data shown below is from an assay that has not been optimized. The coupon was designed for higher flow rates which would decrease the response time and improve the peak shape. Additionally alternative antibody immobilization strategies which could improve the response are currently being investigated.

IMPACT/APPLICATIONS

The oceanographic community does not currently have the capability to make routine and sustained biogeochemical measurements, *in situ* and autonomously, at the same space and time scales that are possible for temperature, salinity, oxygen, and chlorophyll fluorescence. In recent years, though, there has been significant progress in the development and application of reagent-based optical chemical sensors. The on-going research for this NOPP project is giving us the opportunity to further develop, improve and demonstrate these autonomous chemical sensing technologies. These efforts represent substantial advancements in the development of this technology and bring us much closer to a demonstrated capability for sustained, autonomous ocean observations of biogeochemical distributions and variability.

RELATED PROJECTS

WET Labs, Inc., SubChem Systems, Inc. and other partners also have a FY08 NOPP project "Long-term in situ chemical sensors for monitoring nutrients: phosphate sensor commercialization and ammonium sensor development".

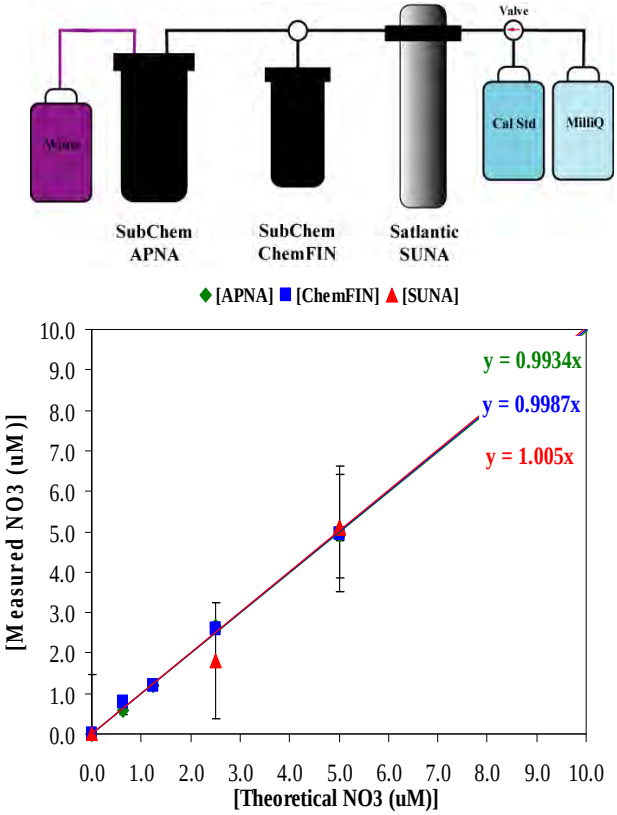
PUBLICATIONS

Veitch, S.P., R. Fratantonio, P. Egli, Charles. P.T., A.A. Adams, J.R. Deschamps, A.K. Hanson and A.W. Kusterbeck. 2011. "Submerged Explosives Detection Platforms using Immunosensing Technology" IEEE-HST Peer Selected Paper on Technologies for Home Security. In Press.

Charles. P.T., A.A. Adams, J.R. Deschamps, S.P. Veitch, A.K. Hanson and A.W. Kusterbeck. 2011 "Explosives detection in the marine environment using UUV-modified immunosensor", Proc. SPIE 8018, 80181U (2011); doi:10.1117/12.883243.

Figure 1. SubChem Systems's compact design for ChemFIN™ the next generation chemical sensing payload for AUVs, Gliders and Profilers. The ChemFIN™ is designed as an independent compact payload containing a micro-fluidic chemical analyzer that minimizes the power and space demands on the deployment platform (AUV, Glider, Profiling Mooring).

[The ChemFIN Nitrate Analyzer is a compact dual-channel fast-response analyzer that can continuously monitor and report the concentration of two nutrients, dissolved nitrate and dissolved nitrite, in real-time. It is designed for profiling or fixed-depth measurements and is readily integrated with CTD systems (i.e. SeaBird Electronics - middle photo).]



Instrument Intercalibration Results

12/17/2008

Instrument ID :	APNA	ChemFIN	SUNA	
Analyte	NO3	NO3+ NO2	NO3	
Sample Media	MilliQ	MilliQ	MilliQ	
Unfiltered Data				
Signal Noise (1/m)	0.348	0.042	1.466 (uM)	= 1 Std Dev
Sample Noise (1/m)	0.387	0.086	1.462 (uM)	= 1 Std Dev
MDL (uM)	0.302	0.044	4.398	= 3 Std Dev
MU (uM)	0.337	0.089	4.386	= 3 Std Dev
UDL (uM)	14.152	84.714	< 100uM	= 10% Light
60s Butterworth Filtered Data				
Signal Noise (1/m)	0.095	0.036	0.403 (uM)	= 1 Std Dev
Sample Noise (1/m)	0.117	0.060	0.370 (uM)	= 1 Std Dev
MDL (uM)	0.083	0.038	1.208	= 3 Std Dev
MU (uM)	0.103	0.063	1.109	= 3 Std Dev
UDL (uM)	14.244	84.667	< 100uM	= 10% Light

Figure 2. Nitrate Analyzer Inter-calibration: The 3 panels show the results of a laboratory inter-calibration experiment involving measurements of nitrate standards in coastal seawater with the 1) ChemFIN, 2) APNA and 3) SUNA Nitrate Analyzers.

[The results show that all sensors were accurate, but the reagent-based nitrate analyzers (ChemFIN and APNA) had better precision and lower detection limits than the optical nitrate analyzer (SUNA).]

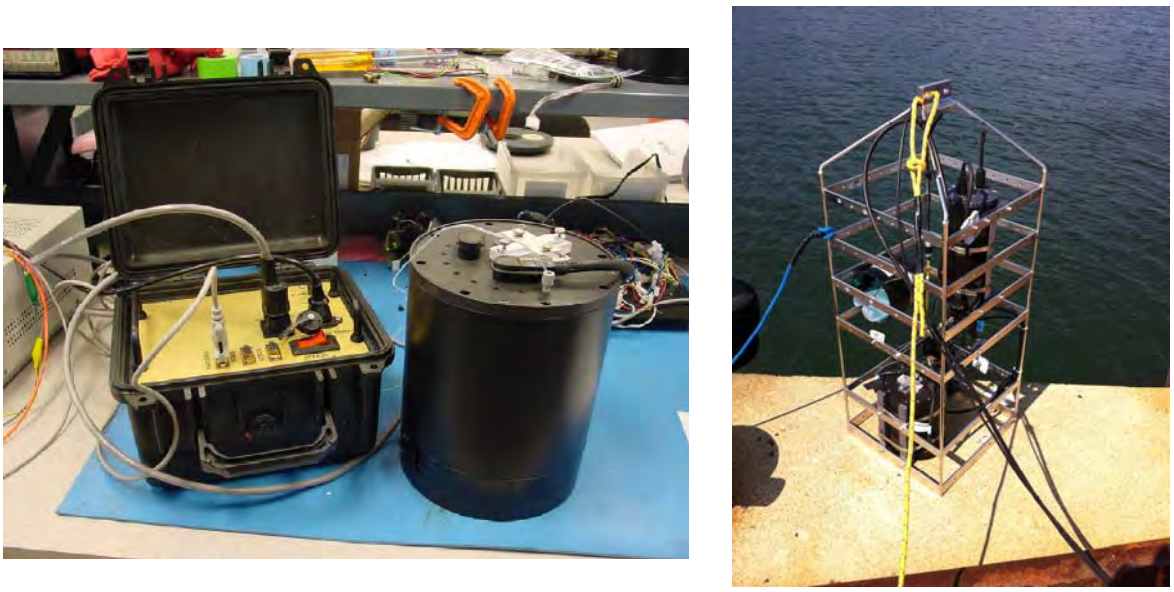


Figure 3. The MarChem Analyzer (left) configured for NRL displacement – based immunoassay with the external integrated face sealing coupon holder and the laser-based fluorescence detection of the CY-5 fluorophore. The system is also shown (right) configured for submerged deployment from the URI-GSO Pier in Narragansett Bay.

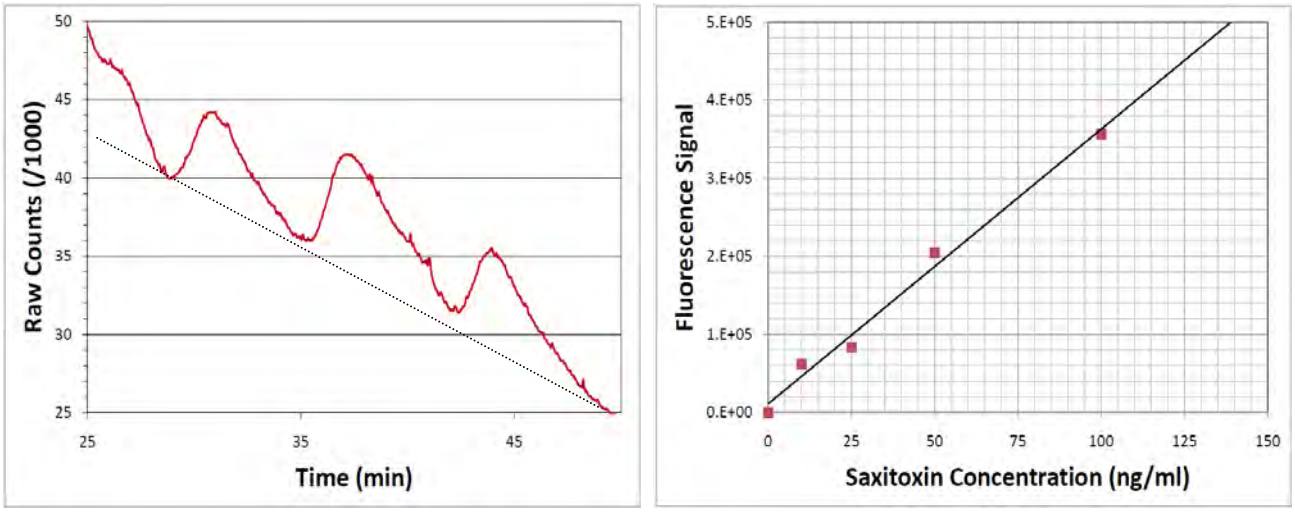


Figure 4. Real time Saxitoxin Analysis; output of flow immunosensor (left) shows gradual decrease in background (approximate position shown by dashed line) and peaks resulting from injection of saxitoxin standard. After integrating the area under each in the concentration range of 0 to 100 ppb saxitoxin the liniear response of the sensor with respect to concentration can be seen (right).